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ORIGINAL ARTICLE

Hemostasis changes during veno-venous extracorporeal membrane oxygenation for respiratory support in adults

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ABSTRACT

BACKGROUND: We investigated the coagulation system in patients during extracorporeal membrane oxygenation (ECMO) initiated for respiratory failure and the influence of the ECMO circuit on coagulation tests; we compared different coagulation tests for monitoring unfractionated heparin (UH) therapy; we investigated whether or not coagulation parameters were predictive of bleeding during ECMO.

METHODS: Pilot study on twelve consecutive adult patients admitted at our general ICU for acute respiratory failure and placed on ECMO from November 2011 to October 2012. Coagulation tests were performed before ECMO start and daily, including day of circuit change and day of circuit removal. UH was monitored with activated partial thromboplastin time (APTT) ratio, at a therapeutic range of 1.5-2.0.

RESULTS: We observed no effect of ECMO circuit on coagulation parameters measured pre- and postlung, but platelet count decreased significantly over time (-82x10^3/mmc, 95%CI 40-123). APTT showed a correlation with antifactor Xa activity, whereas other global coagulation tests such as activated clotting time, thromboelastography and endogenous thrombin potential did not. Major bleeding occurred in three patients but no difference in any coagulation parameter was observed between them and those who did not bleed.

CONCLUSIONS: This pilot study shows that ECMO initiated for respiratory support in adults does not change coagulation parameters. Over time a statistically significant reduction of platelet count was observed, possibly due to consumption within the circuit, consumption microangiopathy or the underlying patients' diseases. Although APTT was appropriate to monitor UH, major bleedings occurred and a lower therapeutic range may be advisable.

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Key words: Extracorporeal Membrane Oxygenation - Blood coagulation - Heparin - Anticoagulants therapy - Partial Thromboplastin Time.

Extracorporeal membrane oxygenation (ECMO) is increasingly being used as an adjunct to conventional ventilatory treatment for respiratory failure.[1, 2] Anticoagulation is required during the whole procedure, to avoid activation of the coagulation system induced by the contact of the blood with the foreign surfaces of both the circuit and the membranes of the artificial lung. Unfractionated heparin (UH) is the anticoagulant of choice during

Comment in p. 147.

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ECMO because of its quickly reversible effect and possible use despite renal function impairment. The laboratory test used in our department to monitor the anticoagulant effect of UH is the activated partial thromboplastin time (APTT), that is highly sensitive to the relatively low UH doses (0.1 to 1.0 IU/mL) used during ECMO.[3] However, patients on ECMO often develop bleeding episodes,[4] sometimes fatal.[5] It is known that the circuit surface, other than the disease leading to ECMO start, causes activation of the contact and complement system [6] and favors the development of an acquired von Willebrand disease.[7, 8] Whether or not bleeding during ECMO is mainly related to excessive anticoagulation, to the derangements of hemostasis caused by the circuit itself or to the underlying patient disease is a matter of debate.

This study aimed to investigate the coagulation system in adult patients during ECMO initiated for respiratory failure, the influence of the ECMO circuit itself on coagulation tests and to compare different coagulation tests for monitoring UH therapy.

Materials and methods

Patients

Consecutive adult patients admitted at our general intensive care unit for acute respiratory failure and placed on ECMO from November 2011 to October 2012 were included in the study. Patients' observation started at admission and ended at discharge from the intensive care unit or at death. Demographic data, causes of respiratory failure, ECMO and ventilatory parameters and transfusions of blood components were recorded daily. Weaning from ECMO, survival at discharge, death during ECMO, death after weaning, bleeding and thrombotic episodes were also recorded. Major bleeding was defined as a fatal one, or intracranial or in other critical area or organ.[9] The day after ECMO removal, survived patients underwent a B-mode ultrasonography to investigate thrombosis of the common femoral or iliac veins, at the site of cannulation. The study protocol and the informed-consent were approved by the ethic committee. Written informed consent or deferred consent was obtained from each patient.

Blood sampling

Blood was drawn from a radial arterial catheter, sampled in vacutainers with 1/10 volume of 3.2% sodium citrate as anticoagulant, centrifugated at 2880 g to obtain plasma that was stored at -80°C. Blood samples were taken before ECMO start (D0) and during ECMO, at four time points arbitrarily defined: D1 was the day of ECMO initiation (at least 5 hours after and within 24 hours from cannulation, following the stabilization of the APTT ratio); D7 was the 7th day after ECMO initiation; CC was the day of circuit change (blood taken immediately before the change); CR was the day of circuit removal (blood taken immediately before removal). As shown in Table I, some coagulation tests were performed daily and at least once. Laboratory data collection started at D0 and ended at CR. To assess the effect of ECMO on the hemostatic system, blood was

TABLE I.—*Time of blood sampling and coagulation tests before and during ECMO.*

Time points	Coagulation tests
Day 0	PT, APTT, fibrinogen, antithrombin, protein C, protein S, factor V Leiden, G20210A prothrombin, antiphospholipid antibodies
Every day	PT, APTT, fibrinogen, antithrombin, activated clotting time, anti-factor Xa activity, thromboelastography, D-dimer
Day 1	Prothrombin fragment 1+2, PAI-1, factor XIII, factor VIII, RiCof, VWF: Ag, ADAMTS13, endogenous thrombin potential
Day 7	
Day of circuit change	
Day of circuit removal	

PT: prothrombin time; APTT: activated partial thromboplastin time; PAI: plasminogen activator inhibitor; RiCof: ristocetin co-factor; VWF:Ag: von Willebrand factor antigen.

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sampled not only from the arterial line in place on the patient, but also from a port placed before and after the artificial lung.

Extracorporeal membrane oxygenation procedure

ECMO was provided by veno-venous bypass through a percutaneous cannulation of both femoral veins in all patients. A 21 to 23 Fr. drainage cannula (HLS cannulae Maquet Cardiopulmonary AG, Hirrlingen, Germany) was extended under fluoroscopy into the inferior vena cava until the renal veins outflow, and a 21 Fr. reinfusion cannula (Bio-Medicus® Femoral Venous Cannulae, Medtronic, Inc.) up to the right atrium. The extracorporeal systems used were the QUADROX PLS Oxygenator plus the ROTAFLOW Centrifugal Pump in nine patients and the HLS Set Advanced 5.0 - Cardiohelp® system in the remaining three (MAQUET Cardiopulmonary AG, Hirrlingen, Germany). In the Cardiohelp® the centrifugal pump is incorporated in the system and placed right in front of the artificial lung. Both systems including oxygenator, centrifugal pump and tubing set, were BIOLINE® coated. An oxygen-air blender was used to ventilate the membrane lung. ECMO was performed under normothermic conditions guaranteed by the oxygenator heat exchanger. The circuit and the oxygenator membrane were inspected thrice daily in order to detect visible clots formed on the membrane surface. Decision to change the circuit was taken considering the presence of visible clots, a sharp increase of D-dimer or a reduction of platelet count, the onset of hemolysis or the reduction of the membrane oxygenating performance. If platelet count decreased rapidly (≥50% from baseline), anti-PF (platelet factor) 4 antibodies for heparin-induced thrombocytopenia were searched.[10]

Laboratory tests

At D0 antithrombin (AT), protein C, protein S, factor V Leiden and G20210A prothrombin gene mutation, antiphospholipid antibodies including lupus anticoagulant, anticardiolipin and anti-ß2 glycoprotein-1 IgG and IgM were tested to investigate inherited or acquired thrombophilia abnormalities. Fibrinolysis was investigated by measuring fibrinogen, D-dimer, prothrombin fragment 1+2 (F1+2), plasminogen activator inhibitor-1 (PAI-1) and factor XIII. Factor VIII (FVIII)/von Willebrand factor (VWF) system was evaluated by measuring FVIII, ristocetin cofactor (RiCof), VWF-antigen (VWF-Ag), and ADAMTS13 metalloprotease activity. All the aforementioned tests were performed as previously described.[11-13]

Anticoagulation monitoring

Anticoagulation with UH started at the insertion of ECMO cannula, with a bolus of 50-70 IU/Kg followed by 10-15 IU/Kg/h in continuous intravenous infusion. UH was monitored with the APTT ratio (patient-to-normal clotting time) aimed at a desired therapeutic range of 1.5 to 2.0, as suggested.[14] The APTT ratio guided the UH dosage. Because the anticoagulant effect of UH is mediated by AT, plasma levels of this protein were measured daily and an AT concentrate was given to the patients when needed, in order to maintain plasma levels above 70%.[14] To calculate the percentage of time with APTT in the therapeutic range, a linear interpolation method was used.[15] In addition to the APTT, the activated clotting time (ACT, sec) and the antifactor Xa activity (UI/mL) were measured, aimed at a desired therapeutic range from 180 to 210 sec according to our institutional protocol and from 0.3 to 0.7 IU/mL,[16] respectively. Antifactor Xa activity is a direct index of inhibition of factor X by UH and is considered to be the gold standard for monitoring the UH anticoagulant effect. ACT (Hemochron® Jr. Signature, ACT-Low Range cartridges, International Technidyne Corporation, Edison, NJ, USA), a point of care test that measures the time of fibrin clot formation in the presence of activators [17]was performed on fresh whole blood according to the manufacturer recommendations.

Two additional global coagulation tests were performed: kaolin-activated thromboelastography (K-TEG) with and without hepa-

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rinase (that neutralizes the effect of UH) and thrombin generation. K-TEG was performed on fresh whole blood (TEG® 5000 Thrombelastograph® Hemostasis Analyzer System, Haemonetics Corporation, Braintree, MA, USA) and among the various parameters recorded the most useful is the time to initial clot formation, conventionally labeled as "R". In case of flat R, *i.e.* inability to form a clot, K-TEG was arbitrarily interrupted at 80 min. Thrombin generation was assessed according to Hemker *et al*.[18] The test was based on the activation of coagulation after addition to plasma of human recombinant tissue factor (1pM) (Recombiplastin, Instrumentation Laboratory, Orangeburg, NY, USA) and synthetic phospholipids (1.0μM) (Avanti Polar, Alabaster, AL, USA) as coagulation triggers. Registration of thrombin generation was obtained with a fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCl, Bachem, Bubendorf, Switzerland) (617μM) by means of a fluorometer (Fluoroskan Ascent™, ThermoLabsystem, Helsinki, Finland). Readings were recorded and calculated with a dedicated software (Thrombinoscope™, Thrombinoscope BV, Maastricht, The Netherlands), which displays thrombin generation curves and calculates the area under the thrombin generation curve, defined as endogenous thrombin potential (ETP). ETP represents the amount of the generated thrombin as a function of the action of pro- and anticoagulant drivers operating in plasma.

Statistical analysis

Categorical variables were expressed as number and percentage. Continuous variables were expressed as mean with standard deviation or median with interquartile range when not normally distributed. Differences in coagulation parameters during ECMO were tested with paired sample t test between D1 and D7 or CC or CR, and between the tube entering (pre-lung) and exiting (post lung) the ECMO circuit. Correlations between coagulation parameters were tested calculating Spearman's rank correlation coefficient (Spearman's rho, ρ) with corresponding P-value. P-value <0.05 was considered as statistically significant. The association between each hemostatic parameter (platelets, fibrinogen and D-dimer) and a measurable K-TEG was tested using unadjusted logistic regression, calculating odds ratios (OR) and their 95% confidence intervals (CI). Statistical analyses were performed using SPSS version 20.

Results

Patients

Twelve patients, 5 men and 7 women, were included in the study and their demographic and clinical characteristics are summarized in Table II. Median age was 69 years (range 31-84). Eight patients started ECMO for chronic obstructive pulmonary disease (including a

TABLE II.—*Characteristics, ECMO duration, transfusion requirement, and outcome of study participants.*

Patient	Sex	Age	BMI	Diagnosis	days on ECMO	Day of circuit change	Major bleeding	RBC transfused (units)	PLTs transfused (units)	FFP transfused (mL)	Outcome
1	M	71	33	COPD	13	Day 2	No	3	-	1500	Dead (day 13)
2	F	31	15	COPD	9	-	No	9	-	-	Dead (day 48)
3	F	63	21	COPD	8	-	No	3	-	-	Discharged
4	F	74	33	COPD	7	-	No	5	-	-	Discharged
5	F	69	27	COPD	20	Day 9	No	10	2	1750	Dead (day 32)
6	F	66	24	COPD	6	-	No	6	-	-	Discharged
7	F	69	20	ARDS	9	-	No	8	2	500	Dead (day 9)
8	M	77	28	COPD	8	Day 5	No	2	-	-	Dead (day 8)
9	M	82	24	ARDS	14	-	Yes[1]	10	1	-	Dead (day 21)
10	F	41	18	ARDS	6	-	Yes[2]	3	7	3750	Dead (day 6)
11	M	84	31	COPD	4	-	Yes[3]	6	1	1500	Discharged
12	M	34	30	ARDS	8	-	No	1	-	-	Discharged

COPD: chronic obstructive pulmonary disease, ARDS: acute respiratory distress syndrome, RBC: red blood cells, PLTs: platelets, FFP: fresh frozen plasma. [1]Retropharyngeal; [2]Intracranial; [3]Retroperitoneal.

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31-year-old woman affected by cystic fibrosis who required a bridging to lung transplantation) and 4 for acute respiratory distress syndrome due to pneumonia in 3 cases and systemic *lupus erithematosous* in one. Median ECMO duration was 8 days (range 4-20) and the circuit was removed before D7 in 4 patients and after D7 in 8. Three patients changed the circuit because of a sharp D-dimer increase above 3500 ng/ml, but no substantial reduction in D-dimer levels was observed after such circuit change. Three patients had a major bleeding event (retropharyngeal, intracranial, and retroperitoneal hemorrhage) during ECMO that was fatal in one, whereas no thrombotic complications were observed nor in the ECMO circuit nor in the eight patients who survived ECMO and underwent ultrasonography. All patients were transfused, for a median number of 6 red blood units per patient (range 1-10). Five patients needed platelet and/or fresh frozen plasma transfusions with a large difference in units required (patient 10 received 50% of platelet units and 40% of fresh frozen plasma volume transfused in all 12 patients, Table II). Seven patients died in intensive care unit, 4 of whom during ECMO, and 5 were discharged. Mean blood flow of the circuit was 2.8±0.6 L/min, revolutions per minute (RPM) 2157±327, gas flow 4,7±2,3 L/min, artificial lung shunt 13±8% and transmembrane pressure drop 13±4 mmHg.

Coagulation changes during ECMO

The comparison of coagulation parameters between D1 and D7 is showed in Table III. Platelet count was the only parameter that significantly changed, with a mean reduction of 82x10^3/mmc (95%CI 40-123) (Figure 1). This reduction was observed in all patients and none of them had heparin induced thrombocytopenia. Six patients required infusion of AT concentrate during ECMO. Of them, three had severe bleeding, and the other three required the change of the circuit. Fibrinogen slightly decreased, while F1+2 and D-dimer increased over time, although not significantly. RiCof and VWF:Ag were high at D1 and the latter increased slightly during ECMO. RiCof/VWF:Ag ratio decreased from D1 to D7, indicating a possible loss of high-molecular-weight VWF multimers. ADAMTS-13 levels remained low and FVIII levels high during ECMO. The same comparison of coagulation parameters was done between D1 and CC or CR, obtaining similar results and an even more pronounced decrease in platelet count (117x10^3/mmc [95% CI 70-165]) than that observed between D1 and D7 (Table IV). FVIII decreased significantly between D1 and CC or CR, but remained always within the normal range. Table V shows the comparison of coagulation parameters between pre- and post-ECMO at D1. No statistically significant difference was observed in any

TABLE III.—*Comparison of coagulation parameters between day 1 (D1) and day 7 (D7) of ECMO.**

Parameter	Normal values	D1 Mean (SD)	D7 Mean (SD)	Difference (95%CI)	P-value#
Antithrombin (%)	70-150	75 (30)	84 (32)	9 (-14 to 31)	0.399
Platelets (10^3/mmc)	130-400	212 (66)	130 (68)	-82 (-123 to -40)	0.002
Fibrinogen (mg/dL)	140-400	379 (182)	348 (103)	-31 (-181 to 119)	0.636
F1+2 (pM/L)	86-128	821 (420)	847 (342)	26 (-431 to 483)	0.897
D-dimer (ng/mL)	0-230	2568 (2346)	3198 (1693)	630 (-1419 to 2679)	0.491
PAI-1 (ng/mL)	<5	0.74 (0.44)	0.93 (0.74)	0.19 (-0.60 to 0.98)	0.592
Factor XIII (%)	70-140	53 (20)	59 (23)	6 (-11 to 23)	0.344
Factor VIII (%)	50-150	244 (65)	247 (46)	3 (-57 to 63)	0.902
RiCof (U/dL)	41-168	331 (204)	334 (208)	3 (-62 to 68)	0.927
VWF Ag (%)	40-169	386 (105)	438 (93)	52 (-33 to 137)	0.193
RiCof/VWF Ag	>0.7	0.84 (0.39)	0.72 (0.24)	-0.11 (-0.25 to 0.02)	0.084
ADAMTS13 (%)	42-150	48 (12)	47 (19)	-1 (-9 to 6)	0.687

*Calculated on 8 patients; #P-value for paired sample *t*-test.
F 1+2: prothrombin fragments 1+2; PAI: plasminogen activator inhibitor; RiCof: ristocetin cofactor; VWF:Ag: von Willebrand factor antigen.

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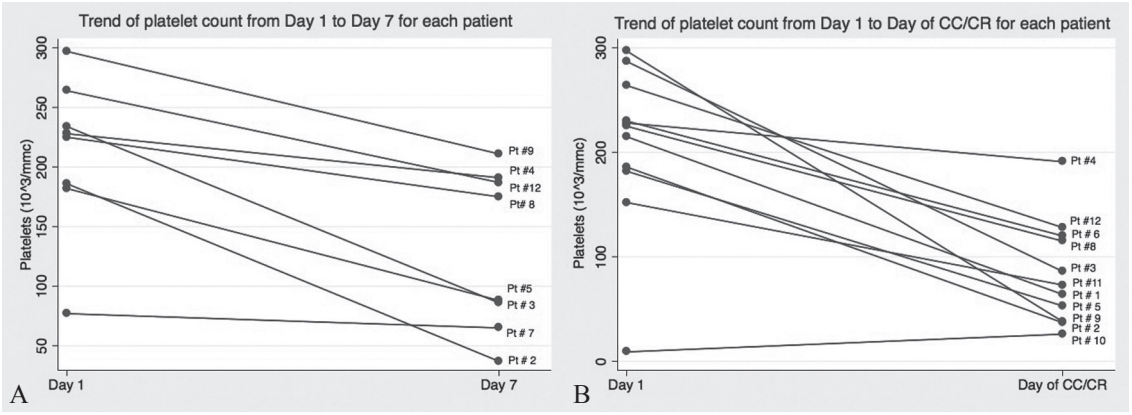


Figure 1.—Trend of platelet count from day 1 to day 7 (panel A) and from day 1 to day of CC/CR (panel B) for each patient.

TABLE IV.—*Comparison of coagulation parameters between day 1 (D1) and day of circuit change (CC) or circuit removal (CR).**

Parameter	Normal values	D1 Mean (SD)	CC/CR Mean (SD)	Difference (95%CI)	P value #
Antithrombin (%)	70-150	69 (34)	83 (29)	14 (-2 to 30)	0.084
Platelets (10^3/mmc)	130-400	202 (75)	85 (50)	-117 (-165 to -70)	0.0001
Fibrinogen (mg/dL)	140-400	409 (143)	330 (138)	-79 (-191 to 32)	0.147
F1+2 (pM/l)	86-128	788 (450)	1098 (275)	310 (12 to 607)	0.043
D-dimer (ng/mL)	0-230	2311 (2159)	4467 (1830)	2157 (223 to 4091)	0.032
PAI-1 (ng/mL)	<5	1.3 (1.6)	0.8 (0.8)	-0.5 (-1.7 to 0.7)	0.386
Factor XIII (%)	70-140	56(14)	59 (20)	3 (-10 to 16)	0.633
Factor VIII (%)	50-150	254 (51)	211(58)	-43 (-81 to -4)*	0.033
RiCof (U/Dl)	41-168	272 (87)	277 (109)	5 (-68 to 78)	0.875
VWF Ag (%)	40-169	393 (98)	412 (123)	19 (-77 to 115)	0.658
RiCof/VWF Ag	>0.7	0.69 (0.13)	0.68 (0.12)	-0.02 (-0.13 to 0.1)	0.744
ADAMTS13 (%)	42-150	44 (17)	42 (19)	-2 (-10 to 7)	0.692

*Calculated on 11 patients, data for Pt. 7 at CR are missing; # P-value for paired sample *t*-test.
F 1+2: prothrombin fragments 1+2; PAI: plasminogen activator inhibitor; RiCof: ristocetin co-factor; VWF:Ag: von Willebrand factor antigen.

TABLE V.—*Comparison of coagulation parameters at day 1 (D1) of ECMO, pre and post-artificial lung.**

Parameter	Normal values	Pre-lung Mean (SD)	Post-lung Mean (SD)	Difference (95% CI)	P value#
Antithrombin (%)	70-150	65 (32)	67 (34)	2 (4 to 0.3)	0.029
Platelets (10^3/mmc)	130-400	-	-	-	-
Fibrinogen (mg/dL)	140-400	424 (141)	412 (124)	-12 (18 to -42)	0.409
F1+2 (pM/L)	86-128	851 (447)	736 (439)	-15 (4 to -34)	0.111
D-dimer (ng/mL)	0-230	2005 (1856)	1953 (1808)	-53 (39 to -145)	0.231
PAI-1 (ng/mL)	<5	1.3 (1.5)	1.2 (1.4)	-0.1 (-0.01 to 0.1)	0.066
Factor XIII (%)	70-140	53 (18)	53 (18)	0 (-2 to 2)	0.856
Factor VIII (%)	50-150	255 (54)	257 (57)	3 (17 to -12)	0.629
RiCof (U/dL)	41-168	293 (156)	297 (149)	5 (16 to -6)	0.38
VWF Ag (%)	40-169	384 (99)	377 (104)	-8 (-1 to -14)	0.027
RiCof/VWF Ag	>0.7	0.75 (0.28)	0.78 (0.25)	0.03 (0.01 to -0.05)	0.037
ADAMTS13 (%)	42-150	-	-	-	-

Calculated on 12 patients; #P-value for paired sample *t*-test
F 1+2: prothrombin fragments; PAI: plasminogen activator inhibitor; RiCof: ristocetin co-factor; VWF:Ag: von Willebrand factor antigen.

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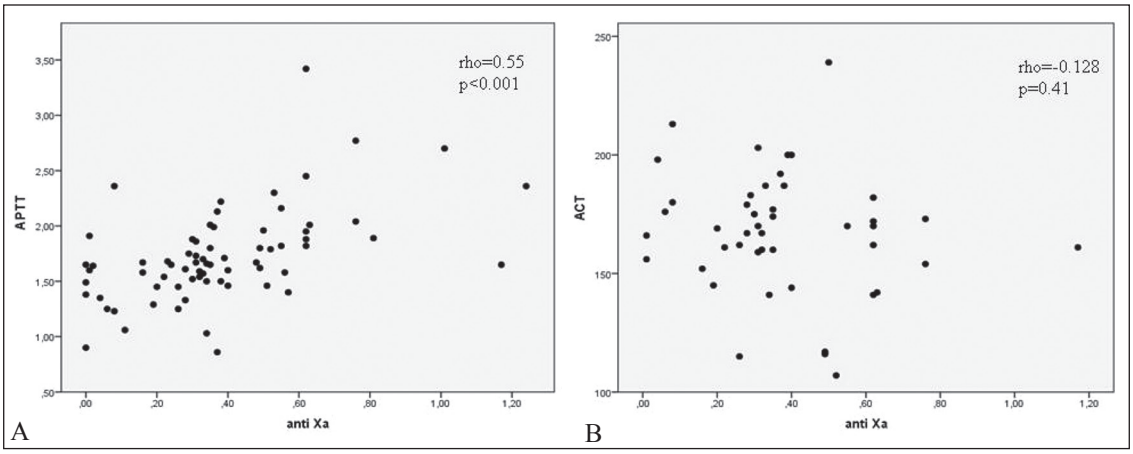


Figure 2.—Correlation between APTT (panel a) or ACT (panel b) and antifactor Xa activity.

parameter, and the same was also at D7 and CC or CR (data not shown). The three patients who had a major bleeding at CC or CR had lower VWF-Ag, fibrinogen and platelet count than patients who did not bleed, but none of these parameters was reduced significantly.

Comparison of different anticoagulation monitoring methods

At D0 the APTT ratio was within the normal range in all patients, and no patient showed thrombophilia abnormalities. The mean APTT ratio during the study was 1.7 (IQR: 1.45-1.89) with 56% (40/71) of samples within and 20% (14/71) above the desired range, resulting in a mean time in therapeutic range of 76%. Median UH infusion rate was 15.2 IU/kg/h (IQR: 12.6-17.4). ACT and antifactor Xa activity were measured in 46 and 70 samples, respectively. ACT showed a mean of 176 sec (IQR 150-179) and a rate of samples within the desired range of 22% (10/46). Antifactor Xa activity showed a mean of 0.38 IU (IQR 0.23–0.52) and a rate of samples within the desired range of 57% (40/70). The correlation between APTT and ACT with antifactor Xa activity is shown in Figure 2. While APTT and antifactor Xa activity were significantly correlated (ρ 0.55, P<0.001), no correlation was observed between ACT and antifactor Xa activity (ρ -0.128, P=0.41). K-TEG showed a flat R at 80 min in 56% of samples (87/154). Only one patient (number 11 in Table II) always formed fibrin within 80 min, whereas the other 11 had at least 40% of samples interrupted at 80 min. When the analysis was restricted to the non-interrupted samples, a correlation between the R parameter and antifactor Xa activity (ρ=0.59, P=0.002) or APTT (ρ 0.45, P<0.001) was observed. Platelet count was the only hemostatic parameter predicting the probability to generate the R within 80 min (for every 10000 platelet increase OR 1.06, 95%CI 1.02–1.11, P=0.009). When K-TEG was performed adding heparinase to the test system, R values were always within 80 min (median 9.8, IQR: 7.3-11.7 min), thus confirming that UH was responsible for the observed results. As expected during UH therapy, thrombin generation measured with ETP was severely reduced in the vast majority (73/90, 81%) of tested samples The ETP lag time was extremely sensitive to the concentration of the reagents. When recombinant tissue factor was increased five times and synthetic phospholipids four times compared to normal values, the lag time decreased from 70 minutes (IQR: 30.5-70.0) to 36.1 minutes (IQR: 20.7-70.0), and when recombinant tissue factor was increased twenty times and synthetic phospholipids four times compared to normal values, lag time decreased further to 5.3 minutes (IQR: 3.2-47.0) and EPT increased from 0 nM*min to 650 nM*min (IQR: 0-1476)”

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Discussion

ECMO support is increasingly used not only as a rescue therapy for acute respiratory distress syndrome, but also in other conditions determining acute respiratory failure. Unfortunately, patients on ECMO still have a high mortality rate due not only to the severe underlying respiratory disease and its related complications, but also to bleeding episodes. In our series of twelve patients seven died, one of them because of intracranial bleeding. The risk of bleeding may be increased by UH, that is necessarily given to patients on ECMO to prevent clot formation in the circuit. Except one study in the early 90's [19] and a recent retrospective study [20] on d-dimer change in adults requiring veno-venous ECMO for ARDS, the majority of the studies investigating the coagulation system during ECMO have been carried out in pediatric patients, who have an immature hemostatic system [21-25] and peculiarities on heparin pharmacodynamics.[26] This pilot study investigated adult patients and aimed to answer the following questions: 1) is there a coagulation consumption in patients during ECMO?; 2) does the circuit itself cause changes in coagulation tests?; 3) if any, are these coagulation changes responsible for bleeding episodes in patients on ECMO?; and 4) is the APTT an accurate test to monitor the anticoagulant effect of UH, compared to other global coagulation tests? Our study shows that the only parameter that changed significantly during ECMO, *i.e.*, until the 7th day of the procedure or the day of circuit change or the day of circuit removal, was platelet count that decreased in all patients. However, only one patient (Pt. 10) had a very low platelet count (<30 *10^3/mmc,) refractory to platelets transfusion. This might be one of the causes of the bleeding diathesis in that patient Having excluded heparin-induced thrombocytopenia, the explanations of this finding may be the underlying infectious conditions, a consumption within the circuit and also a consumption microangiopathy. The latter is surmised upon the observation of the decrease of RiCof/VWF:Ag ratio, possibly due to consumption of high-molecular-weight VWF multimers mimicking an acquired VW disease, as already reported in patients on ECMO.[7, 8] Unfortunately, due to the low number of patients, we were unable to investigate if these coagulation changes played a role in the three major bleeding events observed in our study, and no conclusion can be drawn on their clinical relevance. Concerning monitoring the anticoagulant effect of UH, we observed a correlation between APTT and antifactor Xa activity that measures the direct inhibition of UH on factor Xa and is therefore considered the gold standard to monitor UH anticoagulant effect. The suggested therapeutic range of APTT [14] corresponded to that of antifactor Xa activity in the majority of samples. At variance, the two point-of-care coagulation tests ACT and K-TEG, attractive alternatives to APTT for their easy employment at patient's bed and quick attainment of the results, as well as the most sophisticated thrombin generation test, turned out unsuitable to monitor UH. ACT was successful in monitoring UH during extra-corporeal circulation for cardiopulmonary bypass,[27] despite a limited range of accuracy [28] and the sensitivity to hemodilution, platelet dysfunction and temperature,[29] but not in monitoring UH therapy in pediatric patients on ECMO.[24, 30] Failure of K-TEG, that showed no fibrin nor clot formation for 80 min in more than half of the samples, can be explained by low platelet count but mainly by an oversensitivity of the test to UH concentration, because after addition of heparinase all measurements of K-TEG reverted to normal. A heparin-like effect [31] that may justify the prolonged R values at K-TEG cannot be excluded and may have contributed to the high rate of flat-line TEG. Thrombin generation was totally inhibited, even at APTT or antifactor Xa values below the therapeutic range, indicating that thrombin generation tests when performed at low levels of tissue factor and phospholipids are too sensitive to the anticoagulant effect of UH. Nevertheless the correspondence between the high number of uncoagulable samples at global hemostatic tests (TEG and ETP) despite normal/low range of heparin infusion and antifactor Xa activity, and the high prevalence of

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severe bleeding (25%), let us hypothesize that a further reduction of the dosage of heparin guided by global hemostatic tests could be a line of research.

Several limitations of this study need to be addressed. First, we performed a pooled analysis of a dozen patients with different clinical characteristics and disease severity. Second, the descriptive nature of our study and the small number of investigated patients makes difficult to distinguish the effects of ECMO from those of the underlying disease on coagulation and does not allow definite considerations. For example, measurements pre- and post-lung were restricted to coagulation parameters and platelet count was not performed. Although no effect of ECMO on the former was observed, a selected platelet consumption by the circuit, although unlikely, cannot be ruled out. Third, whether or not the increase in D-dimer levels is a good parameter for the decision to change the circuit remains uncertain. Fourth, some patients received fresh frozen plasma and platelets transfusions that might have influenced the concentration of coagulation factors and tests. Finally, no association between coagulation parameters and the risk of bleeding was observed in our series of patients but again, the small sample size is a major limitation. We can speculate that the APTT therapeutic range, despite its acceptable correlation with antifactor Xa activity, should be lowered as already suggested by others.[32] Moreover platelet function is impaired in patients with infections,[33] but it has not been investigated in this study.

Conclusions

During ECMO initiated for acute respiratory failure coagulation tests do not change but platelet count decreases. The APTT used to monitor UH anticoagulation showed a satisfactory correlation with the antifactor Xa activity, whereas other global coagulation tests did not. However, the clinical relevance of these observations on the risk of bleeding in patients on ECMO as well as a decrease of the APTT therapeutical range remain to be evaluated in further investigations.

Key messages

— The ECMO circuit is supposed to interfere with the coagulation system of patients. In our study no difference in coagulation parameters was observed before and after the artificial lung during ECMO.

— Platelet count decreased significantly over the ECMO days.

— We compared different coagulation tests to monitor heparin therapy. The aPTT was the only test which correlated with antifactor Xa activity. Global coagulation assays such as thromboelastography and endogenous thrombin potential were too sensitive to be suitable to monitor the effect of heparin during ECMO.

— Major bleeding occurred in 25% of the patients. We could not find a relation between coagulation parameters and bleeding.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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